

## (12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization International Bureau



PCT

(10) International Publication Number  
WO 2004/044239 A1

(51) International Patent Classification<sup>7</sup>: C12Q 1/68 (74) Agents: DE CLERCQ, Ann et al; De Clercq, Brants & Partners, E. Gevaertdreef 10a, B-9830 Sint-Martens-Latem (BE).

(21) International Application Number: PCT/EP2003/012058 (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YA, ZA, ZM, ZW.

(22) International Filing Date: 30 October 2003 (30.10.2003) (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data: 02447204.5 30 October 2002 (30.10.2002) EP 0447204.5 30 October 2002 (30.10.2002) EP  
60/440,688 17 January 2003 (17.01.2003) US 60/440,688 17 January 2003 (17.01.2003) US

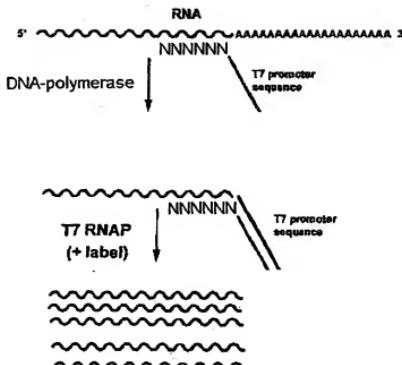
(71) Applicant (for all designated States except US): PAM-GENE B.V. [NL/NL]; Burgemeester Loeffplein 70a, NL-5211 RX Den Bosch (NL).

(72) Inventor; and  
(75) Inventor/Applicant (for US only): BOENDER, Piet [NL/NL]; Ubbergeweg 58, NL-6522 KJ Nijmegen (NL).

Declaration under Rule 4.17:  
— of inventorship (Rule 4.17(iv)) for US only

[Continued on next page]

## (54) Title: IMPROVED METHODS FOR GENERATING MULTIPLE RNA COPIES



(57) Abstract: The present invention is directed to a novel method of efficiently synthesizing, in a non-specific manner, multiple copies of a target RNA. The present invention also relates to kits relating to the same and the use of these copies for determining gene expression pattern. In particular, the present invention relates to a method for generating multiple RNA copies comprising the steps of (a) providing a sample comprising target RNA; wherein said sample is simultaneously contacted with an oligonucleotide comprising at its 5' side a promoter sequence recognized by an RNA polymerase, wherein each oligonucleotide further comprises a target hybridising sequence, which is a random sequence or a predetermined sequence and possibly a modification at its 3' terminal end in such a way that extension therefrom is prohibited; and, an enzyme having DNA polymerase activity; an enzyme having RNase H activity; an enzyme having RNA polymerase activity; and, sufficient amounts of dNTPs and rNTPs; and, (b) maintaining the resulting reaction mixture under the appropriate conditions for a sufficient amount of time for the

WO 2004/044239 A1



**Published:**

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*